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ON CERTAIN PROTEID AND ALBUMINOID REACTIONS AND THEIR SIGNIFICANCE. By J. W. PICKERING, B.Sc.

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ON CERTAIN PROTEID AND ALBUMINOID REACTIONS AND THEIR SIGNIFICANCE.

By JOHN W. PICKERING, B.Sc. (*Lond.*), *Assistant Demonstrator in Biology at St Bartholomew's Medical School.*

(*From the Physiological Laboratory of King's College, London.*)

The experiments about to be described were begun with the object of determining if further modifications in the known tests for proteids would lead to distinctive reactions, and if possible to throw some light on the molecular groups upon whose existence, both the older reactions, and those about to be detailed depend.

My paper can be divided into the following sections:

I. That dealing with previous work.

II. The reactions obtainable with salts of cobalt, nickel, and copper with proteids and albuminoids.

III. The reactions of the same salts with certain proteid decomposition products, and allied substances.

IV. Remarks on the significance of the previous observations.

V. On the significance of the reactions associated with the names of Liebermann, Adamkiewicz, and Millon.

VI. On the xanthoproteic reaction.

VII. On the sulphomolybdic reaction.

VIII. On the saliphonic reaction.

IX. On certain albuminoid reactions.

X. On Krasser's alloxan reaction.

XI. On certain reducing reactions.

XII. On the reactions of proteid precipitates.

XIII. Is there any molecular group associated with coagulation?

XIV. Summary of results.

I. Previous Work.

Piotrowski[1] discovered that the addition of copper sulphate and caustic potash to a proteid gave a violet colour while "peptone" with the same reagents yielded a rose-red solution.

G. Wiedemann[2] found that biuret with these reagents gave a rose-red solution. Hence the rose-red colour reaction of albumoses and peptone is often known as the "biuret reaction."

Brücke[3] laid stress on the difference of the violet colour obtainable with proteids and the rose-red reaction of peptones but considered the radicle which causes the reaction to be in both cases the same, and that some intra-molecular change is the cause of the variation in tint.

As early as 1821 Rudolphi[4] wrote "the components of the dead and living body are not existing under the same chemical conditions," but it was not until 1875 that Pflüger[5] put forward his theory to explain the difference, stating that the assimilation of proteids is probably due to the formation of ether-like combinations between the molecules of the living and the isomeric molecules of the food proteid, water being at the same time eliminated. He also thought that non-living proteids contain the radicle amidogen (NH_2) which is changed into the radicle cyanogen (CN) when they pass into the living condition. He pointed out that uric acid, creatine, guanine, etc. are the products of proteid metabolism and that no such bodies are obtainable from dead proteids.

Loew and Bokorny[6] by experiments on algæ have found that living vegetable protoplasm was able to reduce weak alkaline solutions of silver nitrate, and that dead protoplasm could not. Similar experi-

[1] Piotrowski. *Wiener Acad. Bericht.* Bd. xxiv. S. 335.

[2] Wiedemann. *Poggendorff's Annalen.* Bd. lxxiv. S. 67.

[3] Brücke. Sitzungsbericht. of the Vienna Academy, 1883, reprinted in the *Monatshefte für Chemie.* Bd. iv.

[4] Rudolphi. *Grundriss der Physiologie*, 1821.

[5] *Pflüger's Archiv.* Bd. x. S. 251.

[6] Loew and Bokorny. "Die chemische Kraftquelle im lebenden Protoplasma theoretisch begründet und experimentell nachgewiesen." München, 1882. "Die chemischen Ursachen des Lebens theoretisch und experimentell nachgewiesen." München, 1881. *Pflüger's Archiv.* Bde. xxii. S. 503; xxviii. S. 94; xlv. S. 199. *Maly's Jahresbericht d. Thierchemie.* Bde. x. (1880), S. 3; xi. S. 391—394; xii. 380; xiii. 1; xiv. 349, 474; xvi. 8; xvii. 395. *Biol. Centralbt.* Bde. i. (1881), S. 193; viii. (1888), S. 1. *Bot. Centralbt.* (1889), S. 39. *Journ. f. prakt. Chem.* Bd. xxxi. (1885), S. 129. *Ber. d. Deut. Chem. Gesell.* Bde. xiv. S. 2508; xv. S. 695.

ments on animal protoplasm failed, because of the fatal effect of the reagents upon it. They have put forward the view that proteids were formed by the repeated polymerisation of aspartic aldehyde in presence of a sulphur compound and hydrogen. It has been pointed out that aspartic aldehyde is at present unknown to chemists while Gnezda[1] has shewn that his tests do not correspond to an aldehyde group. Petri[2] has however found proteids react with diazobenzine sulphonic acid and ammonia or a fixed alkali giving a yellow or a red-brown solution dependent on the degree of concentration of the proteid under examination. If these solutions are treated with hydrogen produced by sodium amalgam or by zinc and sulphuric acid an intensely red solution is produced, which is strikingly similar to that formed by an aldehyde or by glucose with the same reagents. Loew has emphasized the fact that proteids when brought in contact with concentrated sulphuric acid yield furfur aldehyde, the presence of which can be demonstrated by the red colour which paper saturated in acetate of xylidene assumes when brought into contact with the solution. For this reason Loew doubts Petri's conclusion while Udranszky[3] has assigned the same reaction to an aromatic group. The existence of an aldehyde group in protoplasm has also been criticized by Baumann[4], who pointed out that Loew and Bokorny's work was open to serious objections, since not only aldehydes reduce alkaline silver nitrate but also pyrogallol, resorin, hydrochinon, pyrocatechnic acid, alloxan and morphine. From these observations they conclude that the reduction obtained by Loew and Bokorny is not necessarily due to an aldehyde radicle. Both Kretzschmar[5] and Griffiths[6] have also independently obtained the reduction with both living and dead protoplasm.

Hlasiwetz and Habermann[7] obtained by chemical decomposition of proteid leucine, tyrosine, aspartic and glutamic acids. Erlenmeyer and Schöffer[8] arrived at the same results by a different method.

[1] Gnezda. *Proc. Roy. Soc.* Vol. XLVII. (1889), p. 208.

[2] Petri. *Zeit. für physiol. Chem.* Bd. VIII. S. 294. Also in *Maly's Jahresbericht*, Bd. XIV. SS. 31, 71.

[3] Udranszky. *Zeit. für physiol. Chem.* Bd. XII. S. 3.

[4] Baumann. *Pflüger's Archiv.* Bd. XXIX. (1882), S. 400.

[5] Kretzschmar. *Biedermann's Centralbt. für Agricultur-Chem.* 1882, S. 830.

[6] Griffiths. *Chemical News.* Vol. XLVIII. p. 179.

[7] Hlasiwetz and Habermann. *Ann. d. Chem. u. Pharm.* Vol. CLIX. p. 304. *Ann. d. Chem. u. Pharm.* Vol. CLXIX. p. 150. *Journ. für prakt. Chem.* (2). Vol. VII. p. 397; also *Anzeig. d. Wien. Akad.* 1872, p. 114 and 1873 Nr. 2.

[8] Erlenmeyer and Schöffer. *Erdmann's Journal.* Vol. LXXX. (1860), p. 357.

Nasse[1] originated the method of investigating proteids by heating them in sealed tubes with barium hydrate and found that the nitrogen of proteids exists in two conditions as evidenced by the fact that one part is in more stable combination than the remainder. The loose parts he thought existed as amides, another portion was combined like the nitrogen of creatine, while the most stable existed as acid amides.

Schützenberger[2] followed up this line of research in many investigations which have lasted from 1875 to the present time. First he confirmed Nasse's results and found that the amount of ammonia and carbon dioxide given off was in the same ratio as if urea had been decomposed. At 200° C. no more ammonia was given and there was a crystalline residuum which contained urea, oxalic, acetic, aspartic, and glutamic acids as well as a dextrin-like body. Next by more careful decomposition he prepared from proteids a body which he has named leucine of formula $x(C_4H_7NO_2)$. He considered that leucine, amido-valeric and oxalic acids together with two bodies which he respectively terms proteic acid and glucoprotein to be the direct molecular decomposition products of proteids. He gave the formula of glucoprotein as $COOHC_nH_{2n}NC_pH_{2p}.COOH$ and considered it was linked to urea or to oxamide by one of its carboxyl groups. Bleunard[3] has confirmed the statements respecting glucoprotein by working with bromine compounds of proteids, while Danilewski[4] states that he has obtained from albumen by careful tryptic digestion a crystalline compound which shews both the reactions of tyrosine and inosite, he however considers that the carboxyl group are united to the metal calcium, since more of that element is found in proteid ash than could be combined with the contained phosphorus.

Schützenberger has obtained both from proteids and gelatin amido acids of the acrylic series and has suggested the understated formula for gelatin[5].

$$C_2O_2 \begin{matrix} \nearrow N(COC_nH_{2n}NHC_mH_{2m}NHC_pH_{2p}COOH)_2 \\ \searrow N(COC_nH_{2n}NHC_mH_{2m}NHC_pH_{2p}COOH)_2 \end{matrix}$$

[1] Nasse. *Chem. Centralblatt.* 1873, p. 137; also *Pflüger's Archiv* (1872), Vol. VI. p. 589, and Vol. VII. p. 139, Vol. VIII. p. 381.

[2] Schützenberger. *Bulletin de la Soc. Chim.* 1875 (15th Feb. 5 Mar. and 15 Mar.); *Ibid.* Vols. XXII. and XXIV.; *Annales de Chim. et de Physique.* 5th series. Vol. XVI. (1879), p. 289; *Comptes Rendus.* CI. p. 1267, CII. p. 289, CVI. p. 1407, CXII. p. 189, (1892). For gelatin CII. p. 1296, also Schützenberger and Bourgeois. LXXXII. p. 262. See *Chem. Centralbt.* 1875, pp. 614, 631, 648, 681, 696, 1876 p. 280, 1877 p. 181.

[3] Bleunard. *Comptes Rendus.* Vol. XC. p. 612 and Vol. XCII. p. 458.

[4] Danilewski. *Archives des Sciences Phys. et Natur.* (Geneva, 1881), Vol. V. p. 107.

[5] Büchner and Curtins have however pointed out that gelatin has the same

He then experimented in the converse manner and synthesized leuceine by heating together in sealed tubes at 160° C. ethylbromide and the zine salts of alanine and glycocoll. Next he heated together at 125° C. proteid decomposition produets and phosphorous pentoxide and obtained a peptone-like body whieh is soluble in water but is precipitated by the following reagents—alcohol, mercuric chloride, Millon's reagent, phosphotungstic, and phosphomolybdie aeids. It also gives with copper sulphate and potassium hydrate a rose-red solution, as well as a marked xanthoproteic reaction. When burnt it emitted the odour eharacteristic of burning animal matter. An objeetion to these views has been urged that urea has not been obtained by ehemieal decomposition of proteids. The old observations of Béehamp[1] and Ritter[2] that when a proteid is decomposed by heating with potassium permanganate it yields urea has been refuted by Städeler[3], Löw[4] and Tappeiner[5] and more recently by Lossen[6] who has shewn that the body which was probably mistaken for urea was guanidine.

Drechsel[7] has however obtained from casein by the aetion of zinc chloride and hydroehlorie acid a body termed by him lysatin which on boiling with baryta water yielded urea.

From a theoretical point of view Latham[8] has assigned to proteids a graphic formula consisting of a benzene nucleus in which is substituted a sulphonic group and a long chain of cyanhydrins (cyanalcohols), the most simple having the formula (CNOH)CH while the most complex has the formula $C_5H_{10}\diagup\!\!\diagdown^{(CNOH)}_{OH}$. The intermediate plaees in the ehain are filled up by cyanhydrins inereasing in eomplexity from the lowest to the highest member of the series. He bases his opinion on the possible syntheses in the organism of many proteid metabolic products from cyanhydrins, and accounts by the means of his formula

percentage composition as amido acrolein and may possibly be a condensation product of that body. (*Bericht. d. deut. chem. Gesell.* Vol. XIX. p. 850.)

[1] Bécamp. *Ann. d. Chem. u. Pharm.* Vol. C. (1856), p. 247.

[2] Ritter. *Comptes Rendus.* Vol. LXX. p. 866, Vol. LXXIII. p. 1323.

[3] Städeler. *Jour. f. prakt. Chem.* Vol. LXXII. (1857), p. 251.

[4] Löw. *Ibid.* Vol. III. (1871), p. 180.

[5] Tappeiner. *Ber. k. Sächs. Gesell.* 1871.

[6] Lossen. *Ann. chem. u. Pharm.* Vol. CI. (1880).

[7] Drechsel. *Ber. d. d. chem. Gesell.* Jahrg. XXIII. (1890), p. 3096.

[8] Latham. *British Med. Jour.* 1886, Vol. I. p. 629 (Apr. 3 and Apr. 10), reprinted as Croonian Lecture by Deighton Bell and Co. Lond. 1887, see also for formation of ptomaines, *Lancet*, 1888 (Vol. II.), p. 751.

for the appearance in the urine of certain pathological products notoriously glucose, uric and hippuric acids, as well as certain ptomaines.

Halliburton[1] has remarked that this view is satisfactory in that it embraces the theories of Pflüger and Löw and I may add that it is in accord with Nasse[2], Kühne[3], and Salkowski[4] who agree as to the existence of an aromatic nucleus in proteids.

Grimaux[5] found that aspartic aldehyde gave a violet tint with copper sulphate and potash and also that the intramolecular anhydride formed by heating by aspartic anhydride with urea to 125° also gave the same reaction.

The most complete investigation on the colour reactions obtainable with caustic potash, copper sulphate with proteids and bodies of allied composition is due to Gnezda[6]. He also found that nickel sulphate and ammonia and potash give yellow and orange reactions respectively with proteids and proteoses (also peptones). Further that certain bodies of simpler constitution and allied to proteids give proteid-like colour reactions with these reagents. These bodies were—uric acid, xanthine, hypoxanthine, biuret, sarcosine, cyanuric and hydrocyanic acids. The following gave negative reactions—glyocine, leucine, tyrosine, ethyl aldehyde, propyl aldehyde, valeryl aldehyde, isobutyl aldehyde, and benzyl aldehyde. From his observations he concludes that the reactions given with the sulphates of nickel and copper with proteids are owing to "the existence in the proteid of cyanogen" while he explains the different colour reactions of proteids and peptones as being due to a different chemical linking of the group cyanogen. The aromatic radicle in proteids has nothing to do with this reaction and he states that hydrocyanic acid approaches very near to peptone in its colour reactions.

Gulkenberger[7] had previously derived hydrocyanic acid, propyl and butyl cyanides from proteids by the oxidizing action of manganese dioxide and sulphuric acid.

Grimaux[8] heated together amidobenzoic acid and phosphorous pentachloride and stated that he has obtained a substance which he

[1] Halliburton. *Chemical Physiology*, p. 117.
[2] Nasse. *Chem. Centralbt.* Vol. x. (1879).
[3] Kühne. *Lehrbuch der Physiol.* 1859.
[4] Salkowski. *Zeit. für physiol. Chem.* Vol. xii. p. 216.
[5] Grimaux. *Comptes Rendus.* Vol. 93 (1881), p. 771.
[6] Gnezda. *Proc. Roy. Soc.* Vol. xlvii. (1889), p. 202.
[7] Gulkenberger. *Liebig's Annalen.* Bd. xliv. S. 39.
[8] Grimaux. *Comptes Rendus.* Vol. 98 (1884), p. 231.

provisionally termed "colloïde amidobenzoïque" and which he believed to be an anhydride formed by the union of several molecules of the acid. Upon drying it appeared as translucent yellow plates which were tasteless, odourless and resembled dried serum albumin. They were soluble in warm water, but became insoluble when heated to 100° C. The insoluble compound thus formed was however soluble in ammonia, sodium phosphate, and alkalis. It was precipitated by hydrochloric, acetic, oxalic, and nitric acids. The precipitate formed by acetic acid was soluble in excess, but gave a flocculent precipitate on the addition of potassium ferrocyanide.

The precipitate with nitric acid dissolves on heating, giving a yellow solution which turns orange upon the addition of an alkali. Excess of calcium hydrate gave a precipitate, but if only $\frac{1}{20}$ of the volume of the solution of calcium hydrate be added, the liquid becomes opalescent and acquires the property of coagulating to a thick jelly when heated.

The coagulation commences at 50° C. when the liquid becomes opalescent, then it becomes milky, and finally, a coagulum is formed between 70 and 80° C. The temperature of coagulation varies with the amount of salt added. Calcium chloride, calcium sulphate, strontium sulphate and barium chloride all react similarly. Also large quantities of sulphate of sodium, acetate of sodium, or acetate of potassium, both retard coagulation, and antagonize the action of the lime salts.

The "colloïde amidobenzoïque" is precipitated by nitrate of mercury, tannin, and by copper sulphate. This last precipitate is soluble in potassium hydrate giving a blue-violet solution. The importance of this research seems to have escaped physiologists, since the phenomena described as characterizing the "Colloïde amidobenzoïque" are identical to those found by Ringer[1] and Sainsbury, by Arthus[2] and Pagès, and by others[3] to be the attributes of proteids. Should these observations be confirmed we can localize the changes of coagulation, and clotting in proteids to molecular changes of their benzene nucleus.

Our knowledge of the aromatic portion of proteids has of late years been greatly advanced by the researches of Salkowski who has investigated the significance of the xanthoproteic reaction, and those of

[1] Ringer and Sainsbury. This *Journal*. Vol. x I. (1890), p. 369.
[2] Arthus and Pagès. *Archives de Physiologie*. T. xxii. (1890), p. 739.
[3] See Halliburton's summary in this *Journal*. Vol. xiii. (1892), p. 831—7.

Adamkiewicz, Millon and Liebermann. Reference to the detail of his work can be more conveniently delayed to the experimental portion of this paper to which we now pass.

II. The Reactions of Nickel, Cobalt, and Copper, Salts with Proteids and Albuminoids.

A. *Positive Reactions.*

I have confirmed Gnezda's tests with nickel sulphate and potash but find that the addition of ammonia to nickel sulphate in the absence of a proteid gives a purplish-blue solution which is but with difficulty distinguished from the pale blue solution given when a proteid is present. Addition of potash to ammoniacal nickel sulphate gives a blue solution which is a marked contrast to the yellow of albumins and the orange of peptones. Gnezda has not recorded the inorganic reactions which are fundamental as a basis to compare with the organic ones. Considering the near chemical relationship of nickel and copper it occurred to me to try if those elements which are next to nickel and copper in Mendelejeff's periodic scheme would give proteid reactions. I find that cobalt which is next to nickel in Mendelejeff's classification gives proteid and albuminoid reactions which are even more distinctive than those yielded by nickel and copper. The salts of iron, manganese and zinc, which are the next nearest elements in the periodic classification do not yield distinctive proteid reactions. Therefore the colour reactions of proteids are not a function of the periodic law[1].

The fundamental inorganic reactions obtainable with cobalt sulphate and the hydroxides of ammonium and potassium are recorded in the following table. In each case very dilute solutions of cobalt sulphate are used[2].

	Ammonium hydrate	Ammonium hydrate + Potassium hydrate	Potassium hydrate alone
Cobalt sulphate	Green solution rapidly changing to yellow with a fine yellow precipitate	Solution changes to blue, with a fine blue precipitate	Solution is colourless, but a well marked bluish green precipitate is formed

[1] Vide Ostwald's *Outlines of General Chemistry*, p. 184, for a recent account of this law.

[2] The most convenient strength is 1 gram cobalt sulphate to 150 c.c. of distilled water.

Since tetraethylammonium hydroxide will be used as a reagent with the sulphates of copper, nickel and cobalt and certain organic bodies I record the colour reactions obtainable from these bodies in the absence of proteids.

Tetraethyl-ammonium hydroxide	Cobalt sulphate	Cobalt sulphate + Potash	Nickel sulphate	Nickel sulphate + Potash
	Blue precipitate changing to green on keeping	Blue precipitate changing to green on keeping	Gelatinous pale green precipitate and a colourless solution	Gelatinous pale green precipitate and a colourless solution
Tetraethyl-ammonium hydroxide	Copper sulphate	Copper sulphate + Potash		
	Pale blue precipitate & a colourless solution	A deeper blue precipitate while the solution remains unchanged		

The following reactions are obtained when egg albumin is introduced.

Egg albumin	Cobalt sulphate	Cobalt sulphate + Ammonia	Cobalt sulphate + Potash	Cobalt sulphate + Ammonium + Potash
	Gives a faint opalescence increasing on shaking	The previous precipitate is dissolved to a clear yellow solution	Heliotrope purple solution and no precipitate	Heliotrope purple solution and no precipitate

Both the heliotrope solutions turn brick-red after standing 30 or 40 minutes. If we use tetraethylammonium hydroxide in the place of ammonia we get with cobalt sulphate and egg albumin a reddish-brown solution and no precipitate. The colour remains permanent for several days and is unaltered by the addition of strong potash.

Copper sulphate tetraethylammonium hydroxide and egg albumin yield a clear violet solution and no precipitate. The colour is also unchanged by potash.

Nickel sulphate and tetraethylammonium hydroxide do not give a distinctive proteid reaction.

Blood serum was prepared free of corpuscles by repeated centrifugalizing and shewed the following reactions,

	Cobalt sulphate + Ammonia	Cobalt sulphate + Potassium hydrate
Serum (pale yellow in tint)	No reaction	First a port-wine colour, on excess of potash a deep heliotrope-purple which changed in 30 or 40 mins. to red-brown

Fibrin was prepared by whipping fresh blood and was repeatedly washed in distilled water until white.

	Cobalt sulphate + Ammonia	Cobalt sulphate + Potassium hydrate
Fibrin	Changes to a dirty brown. On adding potash it reacts as if the ammonia were absent	Purple changing rapidly (i.e. in 30 or 40 secs.) to a purple-brown, then to a red-brown and finally a brown

A mixture of proteoses and peptone was next prepared by dissolving Witte's "peptone" in distilled water and filtering.

	Cobalt sulphate	Cobalt sulphate + Ammonia	Cobalt sulphate + Potash
Witte's "peptone"	Faint opalescence increasing on shaking	Yellow solution and precipitate probably inorganic	Reddish purple, rapidly changing to a red-brown. The changes occupy 5 or 10 secs.

Grübler's and Kanthack's preparations of "peptone" shew the same reaction. The reddish-purple phase lasts a few seconds longer in concentrated solutions of the "peptone" and is absent in dilute solutions. The red-brown is the distinctive reaction.

With cobalt sulphate and tetraethylammonium hydroxide Witte's "peptone" yields a red-brown solution unaltered by potash.

Copper sulphate and tetraethylammonium hydroxide gives with Witte's peptone a rose-pink indistinguishable from that produced by potash. With nickel sulphate and the same bodies we get

	Nickel sulphate + tetraethylammonium hydroxide	
Witte's peptone	Pale greenish yellow precipitate changing in 2 or 3 mins. to an orange precipitate while the solution is pale orange.	On addition of potash to the solution the reaction is unchanged

Peptone free from albumoses was prepared by saturating Witte's

peptone with ammonium sulphate and shaking for four hours. The absence of albumoses was shewn by no precipitate occurring with nitric acid.

	Cobalt sulphate + Ammonia	Cobalt sulphate + Potash
Peptone	Pale yellow solution which turns brown on the addition of potash	Pale brown solution unchanged on keeping. The reaction is more delicate than the copper sulphate reaction

Very dilute solutions of albumen give also a pale brown with cobalt sulphate and potash but Wenz[1] having shewn that ammonium sulphate precipitates all proteids except peptones, this fact does not invalidate the value of the test for minute traces of peptone, which would be missed by the copper reaction, if care be taken to remove all other proteids by the above named method. The order of delicacy of the reagents is, cobalt salts, copper salts, nickel salts.

A nucleo-albumen prepared from brain by Wooldridge's[2] method was kindly put at my disposal by Prof. Halliburton. Nucleo-albumens were stated by Wright[3] not to give the typical violet colour with copper sulphate and caustic potash. I find like Halliburton[4] that they give both this reaction and that of Gnezda typically. They also give the heliotrope purple which characterizes a native proteid when treated with cobalt sulphate and potash.

Acid and alkali albumen were prepared by heating egg albumen to 35° C. for 20 minutes with a few drops of acetic acid, and potash respectively.

Alkali albumen with cobalt sulphate and tetraethylammonium hydroxide gives a heliotrope purple which changes to a red-brown after standing 30 or 40 minutes. Addition of potash does not alter the reaction.

These colour reactions distinguish in a marked manner native proteids from proteoses and peptone the heliotrope purple of the former being absolutely distinctive from the red-brown of the latter.

The reactions depend entirely on the metallic portion of the salt,

[1] Wenz. *Zeit. Biol.* Vol. xxii.

[2] Wooldridge. *Du Bois Reymond's Archiv.* 1886, S. 397.

[3] A. E. Wright. *Brit. Med. Jour.* Sept. 19 and Dec. 19, 1891. *Lancet*, Feb. 27, Mar. 27, 1892.

[4] Halliburton. This *Journal.* Vol. xiii. 1892, p. 819.

since the acetate, sulphate, and nitrate of each of the metals gives the same colour reaction.

Acid albumen	Cobalt sulphate	Cobalt sulphate + Ammonia	Cobalt sulphate + Potash
	No opalescence	Clear yellow solution and no ppt.	Heliotrope purple solution changing after standing 30 or 40 mins. to red-brown solution
Alkali albumen	Cobalt sulphate		Cobalt sulphate + Potash
	Violet ppt. and colourless solution. The precipitate is soluble in ammonia unlike the blue ppt. produced by the addition of potash to cobalt sulphate. In this respect the ppt. resembles the violet ppt. obtained by adding cobalt sulphate and ammonia to either xanthine or hypoxanthine		Heliotrope purple solution changing after 30 or 40 mins. to a red-brown

The presence of either chloride of sodium, sulphate of sodium, or sulphate of ammonium does not affect the reaction. Magnesium sulphate, and sodiomagnesium sulphate upset the reaction.

Pure gelatin was prepared by filtering hot gelatin into alcohol collecting the stringy precipitate redissolving and reprecipitating several times. Finally the gelatin was pressed between glass plates to rid it from the alcohol, was repeatedly washed and finally dissolved in distilled water. It shewed the following reactions.

With nickel sulphate and ammonia it gives either no reaction or a faint bluish colouration which is probably inorganic. On the addition of potash to this solution it changes to a brilliant orange.

With cobalt sulphate it reacts as follows :

Gelatin	Cobalt sulphate	Cobalt sulphate + Ammonia	Cobalt sulphate + Ammonia + Potash (or without the ammonia)
	No precipitate	Faint green to yellowish green solution. This reaction is probably inorganic	A play of colours, commencing with a violet, rapidly changing to a blue, with a faint blue precipitate, then to a green solution, then to a heliotrope purple, then a brick-red and finally to a red-brown. The first four changes occupy about one min., the last two 5 or 6 mins.

It will be noted that the colour play recorded above as taking place with gelatin, cobalt sulphate and potash is in the order of the colours of the spectrum and corresponds to the more stable colours obtained with proteids and peptones.

The colour play is also similar to those colour plays afterwards to be recorded as characterizing certain proteid decomposition products, when treated with these reagents.

	Cobalt sulphate + tetraethyl-ammonium hydroxide	Cobalt sulphate + tetraethyl-ammonium hydroxide + Potash
Gelatin	Green precipitate and solution. A little more of the organic ammonia gives a violet solution, excess a blue	Heliotrope purple solution

Gelatin with copper sulphate and tetraethylammonium hydroxide gives a violet solution unaltered by the addition of potash.

A mixture of gelatinoses and gelatin-peptone (?) was obtained by the action of pepsin and 2% hydrochloric acid on pure gelatin at 35° C. for 72 hours. The solution obtained was straw-coloured and did not gelatinize on cooling. A similar solution was obtained by allowing gelatin to putrefy for 96 hours at 35° C.

Both solutions gave a heliotrope with cobalt sulphate and potash. There was no play of colours. With nickel sulphate of potash they gave a yellow solution, from which a flocculent orange precipitate separates out on standing.

From these solutions gelatin-peptone was prepared by saturating with ammonium sulphate and subsequent filtration. It yielded the same colour reaction as the mixture of gelatinoses and gelatin-peptone.

Keratin was prepared by washing repeatedly white hair (both human and rabbit's) with water and alcohol. No traces of pigment remained. All the alcohol used in the process was removed by washing with distilled water. Treated with copper sulphate and potash, the solution becomes the typical violet, while the solid keratin changes to a dirty brown, probably owing to the excess of potash present. With nickel sulphate and potash the keratin itself becomes a brilliant orange, while with cobalt sulphate and potash it changes to a purple-brown which after standing three or four minutes becomes a red-brown.

Elastin was prepared from ligamentum nuchæ, by soaking small pieces first in boiling water, then in 10% potash, followed by 10% acetic acid. The process occupied fourteen days during which the solutions were repeatedly changed. By this method the proteid and gelatin which is mixed with the Elastin is removed. Like Horbaczewski[1] I find that Elastin gives a typical reaction with copper sulphate

[1] Horbaczewski. *Wien. Acad. Sitzungsbericht.* 1885.

and potash. With nickel sulphate and potash it gives a yellow solution which is best developed after two or three days standing.

With cobalt sulphate and potash it gives a brown solution.

A mixture of elastoses was prepared by digesting with pepsin and 2 % hydrochloric acid for seven days. It shewed a typical "biuret reaction" with copper sulphate and potash, an orange with nickel sulphate and potash, a purple-brown with cobalt sulphate and potash.

The Colloid material in the Thyroid Gland was examined in thin sections cut by a freezing microtome. It yielded typical results of an albuminoid with the three reagents under consideration.

Morochowetz[1] has stated that mucin gives with copper sulphate and potash a blue colouration while Gnezda working with the mucin of vitreous humour finds that with the same reagents it gives a violet, while with nickel sulphate and potash it gives a yellow solution. I have confirmed Gnezda's[2] results with the mucin of vitreous humour, but have found that the most convenient source for mucin is Wharton's Jelly. By extracting finely minced umbilical cord with lime water and precipitating the mucin[3] with an excess of acetic acid comparatively large quantities of that body are obtained. The precipitate should be redissolved and reprecipitated until it is pure. With cobalt sulphate and potash it gave a brown, with nickel sulphate and potash an orange and with copper sulphate and potash a violet solution. If cobalt sulphate be added to egg albumen and the typical heliotrope-purple solution be obtained, then the addition of nickel sulphate changes the solution to orange and the addition of copper sulphate to this solution changes the colour to violet.

Therefore salts of nickel displace salts of cobalt, and these are again displaced by salts of copper in the proteid molecule each in turn yielding its typical colour reaction. Thus the best method for testing for a proteid is to apply the three reactions seriatim to the solution under examination when the changes of colour given will indicate its presence. In a similar manner the gelatin, elastin, and peptone colour re-

[1] Morochowetz. *Petersburg. Med. Wochenschrift.* 1878, No. 6, p. 85.

[2] Gnezda. *Op. cit.* p. 205.

[3] That the body obtained was a mucin was shewn by its giving Millon's and the xanthoproteic reaction as well as forming a reducing substance after boiling with dilute sulphuric acid. According to certain observers the mucin of umbilical cord differs from true mucin in that it contains a higher percentage of nitrogen and of sulphur. Vide *Wächli Journ. für prakt. Chem.* (N.F.). Bd. XVII. (1878), S. 71, and the abstract of Jernström's paper in *Maly's Berichte*, 1880. S. 34.

actions with cobalt salts are displaced by nickel salts, and these again by copper salts yielding in turn their respective colour reactions.

III. The Reactions of the Salts of Cobalt, Nickel and Copper with Certain Proteid Decomposition Products and Allied Bodies.

Biuret which has the known chemical formula of $CO\begin{matrix}\diagup NH_2\\ \diagdown NH\end{matrix}CO\begin{matrix}\diagup NH_2\\ \diagdown\end{matrix}$ reacts with salts of cobalts as stated below.

	Cobalt sulphate + Ammonia	Cobalt sulphate + Ammonia + Potash	Cobalt sulphate + Potash
Biuret	Play of colours commencing at the violet, rapidly changing to blue then a pale claret-red solution and a green precipitate; excess of ammonia gives a yellow and a red-brown solution with a green precipitate	Transient violet changing to blue with a blue precipitate, and a purplish solution. The precipitate changes to green on standing	Transient violet changing to blue solution, then a fine blue precipitate and a purplish solution. The ppt. changes to green on standing

The copper sulphate reaction of biuret is well known as the "Biuret Reaction," while Gnezda has shewn that nickel sulphate with ammonia and biuret gives a blue solution which changes to orange on the addition of potash, in this respect giving the same colour reactions as proteids. Biuret yields negative results with nickel sulphate and tetraethylammonium hydroxide, similar negative results are given with cobalt sulphate and this reagent, it gives however with copper sulphate and the same reagent a pink solution.

Alloxan yields negative results with the sulphates of copper and nickel and the hydroxides of ammonium and potassium. With cobalt salts it reacts as understated.

	Cobalt sulphate + Ammonia	Cobalt sulphate + Ammonia + Potash	Cobalt sulphate + Potash
Alloxan	With traces of ammonia a violet precipitate and a rose-red solution. The precipitate is soluble in ammonia giving a pink solution, which changes redder till it becomes a brilliant orange-red. The violet precipitate when exposed to the atmosphere for 24 hours retains its colour, after 48 hours it becomes a leaden grey	The rose-red solution becomes on addition of potash aheliotrope-purple, excess of potash gives a violet solution	Rose-red solution. If the KOH is added as a few drops, a violet ppt. soluble in potash to a rose-red solution, more potash gives a heliotrope purple, and still more a violet solution

The formula for alloxan is $CO<^{NH.CO}_{NH.CO}>CO$ it being mesoxalylurea (Bernthsen[1]).

Allantoïn gives negative results with the sulphates of copper and nickel and ammonia and potash, also the substitution in these reactions of tetraethylammonium hydroxide for potash gives negative results. With cobalt salts it reacts as below stated.

	Cobalt sulphate + Ammonia	Cobalt sulphate + Potash
Allantoïn	With traces of ammonia a violet, which rapidly changes to a green then to a heliotrope purple, then to a reddish yellow and finally a red-brown solution	If but a trace of potash a violet precipitate, soluble in the slightest excess giving a blue sol. changing to a blue precipitate, which itself changes to green. The solution becomes rose-red

Allantoïn has according to Bernthsen[2] the formula of

$$CO<^{NH—CH—NH}_{NH—CO\quad NH_2}>CO$$

Gnezda[3] found that if a little uric acid was evaporated to dryness on a porcelain dish with nitric acid and nickel sulphate and ammonia added the residuum is coloured deep yellow. If in this reaction we substitute cobalt sulphate for the nickel sulphate the residuum is then coloured a crimson-lake which changes on the addition of potash to a purple-lake.

Uric acid when present in solution (as ammonium or sodium urate) in minute quantities on addition of a trace of cobalt sulphate gives a violet colouration or precipitate dependent on the quantity of uric acid salt present. This reaction is extremely delicate. In larger quantities and dissolved in ammonia it gives on the addition of sulphate of cobalt and a further small quantity of ammonia a dense violet precipitate with a pink solution which becomes redder on standing. The precipitate is soluble in excess of ammonia forming a pink solution which becomes redder on keeping. The precipitate on exposure to the atmosphere turns grey and after twenty-four hours assumes a pinkish-grey hue which remains constant indefinitely. Addition of potash to the solution at any part of its colour play gives a blue solution.

[1] Bernthsen. *A Text-book of Organic Chemistry* (translated by M'Gowan), p. 282.

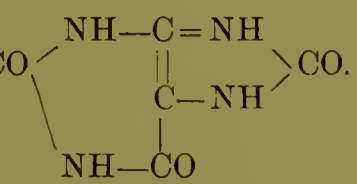
[2] *Ibid.* 283.

[3] Gnezda. *Loc. cit.* p. 207, see also Winogradorff, *Virchow's Archiv*, Bd. xxvii. p. 565, and Worm-Müller, *Pflüger's Archiv*, Bd. xxv. p. 36.

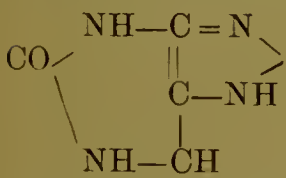
Uric acid dissolved in potash reacts differently as shewn in the following table.

	Cobalt sulphate + Potash	Cobalt sulphate + Potash in excess
Uric Acid in concentrated solution dissolved in potash	A violet precipitate and a colourless solution. The precipitate is soluble in ammonia, giving a faint reddish brown solution. The precipitate on exposure to the atmosphere changes first a deep leaden grey then it becomes greenish, then a deep green, finally after 20 hrs. a paler grey which is constant	A blue ppt. and a colourless solution. The ppt. changes to a dirty green on exposure to the atmosphere and finally after 24 hrs. to a grey which is constant

The dense violet precipitate formed by the addition of cobalt sulphate to uric acid dissolved in ammonia is soluble in tetraethyl-ammonium hydroxide giving a brown solution which turns heliotrope-purple on the addition of potash.

The formula for uric acid as determined by the researches of Medicus and Fischer[1] is  Closely related

to uric acid both as a katabolic product of the organism and chemically is xanthine which has the following formula ascribed to it by E. Fischer[2].

 Xanthine and hypoxanthine have both been

shewn by Gnezda[3] to react similarly to uric acid with the sulphates of Nickel and Copper together with ammonia and potash; with cobalt salts it reacts as understated.

[1] Medicus and Fischer. *Ann. d. Chem. u. Pharm.* Bd. CLXXV. (1875), S. 230, *Ber. d. deutsch. chem. Gesell.* 1884, S. 328, 1785.

[2] E. Fischer. *Annalen der Chem.* Bd. 215, p. 253.

[3] Gnezda. *Loc. cit.* p. 207.

	Cobalt sulphate + Ammonia	Cobalt sulphate + Potash
Xanthine	With traces of ammonia a violet precipitate soluble in more ammonia, giving a greenish yellow sol. which changes to a red brown. The solution obtained by filtering off the violet precipitate is colourless. The precipitate changes a leaden grey on exposure to the air	With traces of potash a transient violet soluble in the slightest excess to a blue solution. If potash be added to the ammonia solution we get a blue solution changing to a blue precipitate which itself becomes green

If tetraethylammonium hydroxide is substituted in the place of ammonia in the cobalt reactions of xanthine we get these reactions:

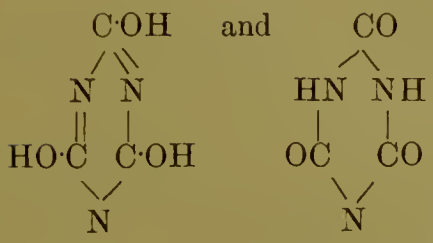
	Cobalt sulphate + tetraethylammonium hydroxide
Xanthine	With traces of the tetraethylammonium hydroxide a violet precipitate insoluble in excess but changing blue and finally greenish when excess is added. The filtrate is colourless. The violet precipitate changes on exposure to the atmosphere to a leaden grey and finally an olive green. Addition of potash to the solution gives the same reaction as recorded in the previous table

Copper sulphate and nickel sulphate yield negative results with xanthine and tetraethylammonium hydroxide.

Hypoxanthine which differs from xanthine in having one oxygen atom less reacts throughout like xanthine differing only in that its violet precipitate remains long permanent in the air but after seventy-two hours changes to a pinkish-grey which is permanent.

Gnezda has pointed out that Cyanuric acid reacts with the sulphates of nickel and copper giving the same colours as are given by proteids with these reagents, and if Gnezda's view that proteids contain a "cyanogen radicle" be correct, we should find that cyanuric acid gives colour reactions more closely allied to those of proteids than are the reactions given by the bodies described above. It must however be borne in mind in considering the reactions of cyanuric acid in its relation to proteid reactions, that cyanuric acid itself is liable to undergo intra-molecular changes forming two series of compounds, so that A. W. Hofmann[1] has assigned two hypothetical formulæ for cyanuric acid which are

[1] A. W. Hofmann. *Berichte der deutschen chem. Gesell.* Vol. XVIII. p. 2755; see also p. 3261.



and shew that its reaction may equally depend on the group CN or on the group CONH. In its cobalt reaction I record these agreements and differences both from proteids and the allied bodies under consideration.

	Cobalt sulphate + Ammonia	Cobalt sulphate + Potash
Cyanuric acid	With traces of ammonia a dense violet precipitate and a marked pink solution, a little more ammonia gives a heliotrope purple, still more a pink which changes to a brick-red, while excess gives a yellow brown solution. The violet ppt. on exposure to the air changes first green and finally grey	If a very small quantity of potash be used we get a dense white precipitate which has a pale pink hue and a colourless solution. The precipitate[1] is permanent in air. If more potash be added the white precipitate changes to a dirty green which is probably an inorganic reaction. The solution is unaffected

It will be noted that the reaction with cobalt sulphate and potash differs markedly from all other reactions recorded.

Gnezda[2] has shewn that the reactions given by hydrocyanic acid with the sulphates of copper and nickel, together with ammonia and potash are identical with those obtained with peptones and albumoses. This he deduces as evidence in favour of a cyanogen radicle in those bodies. Bernthsen[3] remarks that hydrocyanic acid sometimes reacts as its hypothetical isomer C≡NH. Therefore we must not unreservedly accept the view that the similarity of the colour reactions of proteoses and peptone with those of hydrocyanic acid is due to a cyanogen radicle in the former bodies, it might just as likely be owing to an imido group. Further with cobalt sulphate it reacts otherwise.

[1] The precipitate when heated in platinum foil turns a brilliant blue, and finally carbonizes. Heated with Potassium Dichromate and sulphuric acid it evolves carbon dioxide. It is therefore organic.

[2] Gnezda. *Loc. cit.* p. 208.

[3] Bernthsen. *A Text-book of Organic Chemistry* (translated by M'Gowan), p. 253.

Hydrocyanic acid	Cobalt sulphate + Ammonia	Cobalt sulphate + Ammonia + Potash	Cobalt sulphate + Potash
	Greenish yellow solution	Greenish yellow solution	Greenish yellow solution

Hydrocyanic acid	Cobalt sulphate + tetraethyl-ammonium hydroxide	Cobalt sulphate + $N(C_2H_5)_4OH$ + Potash
	A green precipitate soluble in excess forming a greenish yellow solution	A greenish yellow solution

The above reactions differ markedly from the characteristic proteid-like reactions given by biuret, alloxan, allantoïn, etc., which seems to shew that hydrocyanic is not so closely related to the proteid molecule as was supposed.

Murexide was dissolved in water and the typical purplish solution with the reagents under consideration. It reaeted as understated.

Murexide	Copper sulphate	Copper sulphate + Ammonia	Copper sulphate + Potash
	Solution became yellow	Solution became purple, the same as the original solution	Heliotrope purple changing on excess of potash to a violet
Murexide	Nickel sulphate	Nickel sulphate + Ammonia	Nickel sulphate + Potash
	Solution becomes orange	Solution becomes an orange red	Red with an orange hue in it, a ppt. of the potash is in excess
Murexide	Cobalt sulphate	Cobalt sulphate + Ammonia	Cobalt sulphate + Potash
	Solution becomes orange	Solution becomes purple like the original solution	Heliotrope purple changing on excess of potash to a violet which gets redder

If biuret be treated with the salts of cobalt nickel and copper (with ammonia and potash) seriatim, we get the same replacement of one metal by the other in the biuret molecule and the same change of colours as I have recorded in the proteid and albuminoid molecule.

Salts of manganese, iron, and zinc, do not give any colour reactions with the above-named substances, which also behave in this respect like

proteids and albuminoids. The reactions cited above are independent of the acid radicle united to the metals as they are in the proteid reactions.

B. *Negative Reactions.*

Aromatic proteid decomposition products and bodies of allied chemical composition yield negative results as tyrosine, hippuric, plenylacetic, benzoic, salicylic, salicylsulphonic, cholalic, taurocholic and glycocholic acids, phenol, indol, and skatol, I conclude that the reactions obtainable with the salts of copper, nickel, and cobalt, and proteids and albuminoids are unconnected with the aromatic radicle of the proteid or albuminoid.

Similarly they do not depend on the existence of an aldehyde group either aromatic or fatty.

Leucine, asparagine, and glycocine all give negative results, and these being the most common of the amido-acids obtainable from proteids it is probable that the reactions do not depend on their formation or presence. Xanthine and hypoxanthine as previously recorded give typical colour reactions, but if two methyl groups are introduced into the xanthine molecule to form theobromine it is found that this substance gives no colour reactions with the reagents under consideration. Caffeine (*i.e.* trimethylxanthine) also does not react. This shews that a comparatively small difference in the molecule from that of a proteid katabolic product prevents the proteid-like reactions of the body.

Lossen[1] has found guanidin amongst the oxidation products of proteids. This body has the formula $NH=C(NH_2)_2$ (Bernthsen[1]) and gives negative reactions, so also creatine of formula

$$HN=C\begin{matrix}\swarrow NH_2\\ \searrow N(CH)-CH_2\cdot COOH\end{matrix}$$

which is well known as occurring in muscular tissue. Inspection of these formulae shews that the reaction does not depend on the imido group alone.

IV. **On the significance of the preceding Observations.**

There is little doubt that the colour reactions produced with proteids and the salts of cobalt, nickel, and copper, depend on the grouping of the nitrogen in the proteid molecule. The reactions were thought by Gnezda[1] to depend on the radicle cyanogen which as seen in the

[1] *Op. cit.*

historical summary was thought by Pflüger[1] to characterize living protoplasm. Now Gnezda's experiments were all made on non living proteids, hence there is a marked divergence between these two observers. Gnezda[2] bases his opinion chiefly on the fact that cyanuric acid gives with nickel and copper salts the same colour reactions as are obtained with peptones and albumoses. Examined with cobalt sulphate and potash it will be noted that cyanuric acid differs markedly from proteids in its colour reactions, while its resemblance to a proteid in its copper and nickel reactions may be possibly accounted for by its undergoing tautomeric change as mentioned previously. With hydrocyanic acid and cobalt salts only inorganic reactions have been obtained, which again point away from a cyanogen radicle in proteids. Returning to Pflüger's view it will be noticed that the amidogen group occurs in both biuret and allantoïn which give colour play reactions similar to those obtained with gelatin and cobalt salts.

It will be noticed that where the colour plays are deficient in certain portions of the spectrum, they still remain in spectral order, and correspond to the more permanent colour tints given by different proteids. The closest resemblance in colour reactions is between gelatin and biuret xanthine, alloxan, and allantoïn. Inspection of the formulæ of these bodies shews that they all contain the group (CONH), and it seems not improbable that the colour reactions given by proteids and albuminoids with the salts of cobalt, nickel and copper may depend on the molecular group (CONH). The term biuret reaction has recently become restricted to pink colour with copper and potash proteoses and peptone. This term seems to be too narrow in its application since the colour play given by biuret with cobalt salts more resembles that yielded by the albuminoid gelatin than that given with peptone.

On account of the generic position of biuret in all the reactions yielded with the metals under consideration and proteid katabolic products I would venture to suggest that the term "biuret reaction" should only be used in a generic sense.

The copper-proteid reaction might be known as the ionoproteic reaction (from the Greek ἴον=a violet) while the rose-red peptone reaction now known as the biuret reaction could be termed the rhodoproteic reaction (from the Greek ῥόδον=a rose). The view put forward by Latham that proteids contain the group (CNOH) is not at variance with the theory ventured above, since the extremely unstable nature of

[1] *Op. cit.* [2] *Op. cit.* p. 277.

cyanhydrins and the known tautomeric[1] changes of this chemical group would make it probable that in many of their reactions they would play the *rôle* of (CONH); and besides, since Latham's theory is based on percentage calculations, his results are as applicable to my hypothesis as his own. Interesting results may accrue from the application of the nickel and cobalt tests to the peptone-like body that has been recently prepared by Schützenberger.

V. On the significance of the Reactions of Liebermann, Adamkiewicz, and Millon.

(*a*) Liebermann's Reaction[2] which is performed by precipitating albumen with alcohol and washing the precipitate with alcohol and ether and then heating it with strong hydrocloric acid, when a fine blue colour is developed. Würster[3] pointed out that the colour is intensified by the addition of a few drops of sulphuric acid.

Le Nobel[4] states that the reaction is not given by pure peptone. Bodies of the ureid series of proteid katabolic products like uric acid, xanthine, hypoxanthine, alloxan, do not give the reaction. The same applies to tyrosine, leucine, phenol, skatole, salicylic and salicylsulphonic acids. The last two bodies were experimented with since many observers agree in pointing to a hydroxy-benzoic group in proteids. Salkowski[5] goes so far as to state that Liebermann's reaction is not yielded by any of the aromatic members of the putrefaction products of albumen. It will be seen from the above that as far as I have experimented a similar negative result has been obtained. Indole (of Merck's manufacture) however gives a rose-red tint which was distinct from the blue given by proteids.

(*b*) Adamkiewicz' Reaction[6], which can be intensified by adding a crystal of potassium nitrite to the sulphuric and acetic acid used in performing the test, has been shewn by Salkowski to be only given by those putrefactive derivatives of albumen which belong to the indole group. I can fully confirm Salkowski's results since indole and skatole

[1] Thus simple variations of pressures and temperature give us $C_3O_3(NC_2H_5)_3$ normal cyanuric ether and $C_3O_3(NC_2H_5)_3$ tricarbimido ether (Bernthsen, *op. cit.* p. 259).

[2] Liebermann. *Maly's Jahresb.* Bd. XVIII. S. 8, and *Chem. Centralbt.* 1877, S. 600.

[3] Würster. *Maly's Jahresb.* XVIII.

[4] Le Nobel. *Ibid.* XVIII. S. 31.

[5] Salkowski. *Loc. cit.* p. 215.

[6] Adamkiewicz. *Ber. d. deut. chem. Gesell.* Bd. VIII. S. 761.

give the reaction typically while the following bodies yield negative results—allantoïn, xanthine, hypoxanthine, alloxan, biuret, leucine, glycocine, hippuric, cyanuric, hydrocyanic, and benzoic acids, as well as tyrosine.

It is therefore probable that the Adamkiewicz' reaction of proteids is due to the aromatic portion of those bodies, but more we cannot say, since the glacial acetic acid and the sulphuric acid must considerably change the proteid molecule, while its appearance as a putrefaction product associated with certain micro-organisms (Harris and Tooth[1]) renders it probable that indole and skatole are not simply broken off from the proteid molecule, but have undergone changes in being so severed.

(*c*) Millon's reaction[2] which was thought by Kühne[3] to be due to tyrosine. Kühne and Chittenden[4] have found that the anti-products of digestion which do not yield on further digestion, or on decomposition with sulphuric acid either leucine or tyrosine, do not give this reaction. Salkowski[5] also believes the reaction to be due to tyrosine. Nasse[6] has however stated that Millon's reaction is due to benzene derivatives in which one hydrogen atom has been substituted by hydroxyl. Schützenberger[7] has found that leucine and tyrosine are absent from the putrefaction products of gelatin. Salkowski has declared that gelatin does not react with Millon's reagent, but Chittenden and Solley[8] have recently found the product of gelatin digestion which corresponds to antialbuminid to yield a characteristic reaction.

Millon in his original memoir published in 1849 stated that gelatin gave his reaction, and I have found that pure gelatin and gelatinoses prepared therefrom give the reaction in a marked manner. Thus we have albuminoids from which tyrosine is unobtainable giving Millon's reaction.

[1] Harris and Tooth. This *Journal.* Vol. IX.

[2] Millon. *Comptes Rendus.* T. XXVIII. (1869), p. 40.

[3] Kühne. *Zeit. Gesammt. Natur.* (Halle), XXIX. S. 506. *Virchow's Archiv*, Bd. XXXIX. S. 130.

[4] Kühne and Chittenden. *Zeit. Biol.* Bd. XXII. S. 423.

[5] Salkowski. *Zeit. für physiol. Chem.* Bd. XII. S. 215. *Berlin. Klin. Woch.* (1885), Hft. 2.

[6] Nasse. *Chem. Centralblatt.* Bd. X. (1879).

[7] Schützenberger. Article in *Wurtz' Dictionnaire de Chim.* (1886), Supplement 1—A— p. 58.

[8] Chittenden and Solley. This *Journal.* Vol. XII. p. 23.

Dealing now with proteid decomposition products and allied bodies, I have like Nasse found that bodies which contain one hydroxyl in a benzene nucleus give the reaction.

Thus tyrosine, salicylic, salicylsulphonic acid and phenol react typically while benzoic and phenyl acetic acids which contain the aromatic but not the hydroxyl group give negative reactions. Indole yields a terra-cotta coloured precipitate and a yellow solution; skatole an orange coloured precipitate and a yellow solution. Bodies of the nitrogen group of proteid katabolic products such as—creatine, asparagine, alloxan, allantoïn, biuret, xanthine, hypoxanthine, pure leucine, glycocine, uric, hippuric, hydrocyanic and cyanuric acids, all give negative results. The introduction of a second hydroxyl group into the molecule as in pyrogallol also prevents the reaction. Millon's reaction in the case of proteids is therefore doubtless due to an aromatic radicle, but whether it is owing to tyrosine must be left sub judice. The researches of Corvisart[1], Kühne[2], and Sheridan-Lea[3], who have proved tyrosine to be a normal product of proteid digestion, go a long way to shew that a tyrosine-like radicle occurs in proteids; and a possible reconciliation of the views of Kühne and Nasse may be found in the fact that tyrosine itself owes its own Millon's reaction to its hydroxybenzene nucleus since amido acids do not give the reaction. Gelatin though it does not contain a tyrosine radicle probably contains a hydroxybenzene nucleus and hence its reaction.

VI. **The Xanthoproteic Reaction.**

If the so-called xanthoproteic acid which is formed by the addition of ammonia to albumen that has been heated with nitric acid be tested by Millon's reaction we get the ordinary change to red but with the other proteid tests, viz., those of Adamkiewicz, of Liebermann, of Piotrowski, of Gnezda, and with salts of cobalt and the hydroxides of ammonium and potassium only negative results are obtained. The filtrate also obtained in performing the xanthoproteic test gave the same results and further was not precipitated by a saturated solution of salicylsulphonic acid.

O. Loew[4] has urged that the orange precipitate consists of a mixture

[1] Corvisart. *Gaz. hebdomadaire.* T. IV. (1857), Nos. 15, 16, and 17.
[2] *Ibid.* p. 130.
[3] Sheridan-Lea. This *Journal.* Vol. XI. (1890), p. 244.
[4] Loew. *Journ. für prakt. Chem.* Series II. Vol. III. p. 180.

of oxynitro, tri-nitro, hexanitro, and hexamidoalbumin sulphonic acid. From the above observations I conclude that the proteid in performing the xanthoproteic test has become so altered that to term it a nitroalbumin etc. is a misnomer.

Salkowski[1] has shewn that those products of the putrefaction of proteids which are allied to indole and phenol give the xanthoproteic reaction.

The following bodies I find to give the reaction, salicylic acid, salicylsulphonic acid, phenol, cholesterin, cholalic acid, taurocholalic acid. The last three bodies evolve nitrous fumes when heated with nitric acid.

The following bodies yield negative results, glycollic acid, creatine, xanthin, hypoxanthine, all giving pale green solutions, skatol and hydrocyanic acid a pale yellow solution, phenyl-acetic gives a colourless solution, while allantoïn, biuret and benzoic acid give greenish-yellow colourations.

Pure leucine gives a negative reaction but this body often contains tyrosine as an impurity when the reaction is given in a marked manner.

Cyanuric acid gives the yellow on boiling with nitric acid but the yellow remains unchanged on addition of ammonia. Pyrogallol also gives the yellow with boiling nitric acid but forms a brown solution on addition of ammonia, shewing that the introduction of a second hydroxyl group into the benzene nucleus prevents the reaction. Indole gives a yellow with hot nitric acid but on addition of ammonia the solution becomes rose-red which changes on excess of ammonia back to a yellow.

From the above observations it will be noticed that those bodies which have a benzene nucleus with one hydrogen substituted by hydroxyl give the xanthoproteic reaction and that those bodies which contain a benzene nucleus without the hydroxyl as in phenyl-acetic, and benzoic acids do not give the reaction. Hence it is not improbable that the xanthoproteic reaction of proteids and cholalic acid depends on the existence of an hydroxybenzene nucleus.

VII. **The Sulphomolybdic Reaction.**

Fröhde[2] found that a solid proteid when heated with sulphuric and molybdic acids gives a brilliant blue precipitate. I have noted that the blue precipitate forms on heating a brilliant green solution. The precipitate is also soluble in ammonia and deposits a white crystalline

[1] Salkowski. *Zeit. für physiol. Chem.* XII. p. 215.
[2] Fröhde. *Annalen der Chem. u. Pharm.* Vol. CXLV. p. 376.

precipitate when potassium hydrate is added to the ammoniacal solution.

Tyrosine and salicylic acid give blue and violet precipitates respectively but form yellow solutions on the addition of ammonia and deposit yellow crystalline precipitates on the addition of potassium hydrate, allantoïn, creatine, benzoic, cyanuric, and phenyl-acetic acids react in every way like albumin. Biuret however gives a negative reaction. Leucine, guanidine, hypoxanthine and glycocoll all give the blue reaction.

The above experiments shew that bodies both of the aromatic and ureïd groups give the sulphomolybdic reaction which therefore cannot by this method be localized to any particular molecular group. Since bile salts give the reaction it becomes inaccurate when used as has been suggested as test for albumin in the urine.

VIII. **The Saliphonic Reaction.**

Roch[1] suggested the use of salicylsulphonic acid as a test for albumins. McWilliam[2] independently discovered the reaction and has pointed out: (α) That it precipitates out all the proteid from the solution to which it is added. (β) That it reacts with all classes of proteids. (γ) That with albumins and globulins the precipitate coagulates on heating while with proteoses the precipitate disappears on heating and reappears on cooling. (δ) That even peptone can be precipitated from a solution saturated with ammonium sulphate. The reaction has the disadvantage that the precipitate is white and until all bodies that might occur in a pathological urine have been tried cannot be elevated to an absolute test for albumin in the urine. Since the reaction is extremely delicate it is of practical importance to make the test indisputable, and this becomes more necessary since the remaining tests for proteids are liable to react with substances other than albumins. The following modification of the test may enhance its accuracy. The precipitate obtained from the solution after the addition of the salicylsulphonic acid should be repeatedly washed with distilled water to remove all traces of the acid itself. The precipitate then shews the following reactions[3]:

[1] Roch. *Pharm. Centralhalle*, 1889 (Sept. 19th), p. 549.

[2] Mc William. *British Medical Journal*, 1891. Vol. I. p. 837, also 1891, Vol. I.

[3] Salicylsulphonic acid itself does not react with the tests of Liebermann and Adamkiewicz. With copper sulphate and potassium hydrate it gives a brilliant green solution while with the nickel sulphate and cobalt sulphate tests it gives negative reactions.

(1) Turns yellow on heating with nitric acid, changing to orange on the addition of ammonia.

(2) Treated with alcohol and ether, and then heated with strong hydrochloric acid it turns a brilliant blue (Liebermann's reaction).

(3) Dissolves in glacial acetic acid and on the addition of a few drops of sulphuric acid yields a fluorescent violet (Adamkiewicz reaction).

(4) With copper sulphate and potash gives the typical violet.

(5) It gives Millon's reaction typically.

(6) It gives the typical yellow with nickel sulphate and potash.

(7) It gives a typical purple-brown with cobalt sulphate and potash.

The precipitate given by salicylsulphonic acid therefore yields all the chief colour reactions of a proteid precipitate.

Mucin as has been pointed out by McWilliam might be confused in a urine analysis with a proteid, since that body is precipitated by salicylsulphonic acid. McWilliam relies on the physical peculiarities of mucin as a safeguard against this confusion. This however in the hands of an inexpert observer might lead to error. Mucin moreover as shewn by Gnezda and corroborated in this paper gives a typical reaction with salts of copper and nickel. It also gives all the other reactions cited above. All doubt is however obviated by boiling a small portion of the precipitate with dilute sulphuric acid, and testing its reducing properties with Fehling's solution.

Salicylsulphonic acid precipitates gelatin, the precipitate disappears on heating and reappears on cooling in this respect resembling a proteose, but on further cooling gelatinization sets in, shewing that gelatin like a proteid undergoes comparatively little change when precipitated by salicylsulphonic acid. Elastoses are precipitated by salicylsulphonic acid, the precipitate disappears on heating and reappears on cooling.

The following bodies related to proteids are not precipitated by saturated salicylsulphonic acid—alloxan, biuret, indol, skatol, phenylacetic acid, uric, glycollic, cholalic, pure glycocholic and taurocholic acids[1].

I have therefore failed to find any body related to proteids or albuminoids that is precipitated by salicylsulphonic acid.

[1] Commercial preparations of bile salts contain mucin and therefore precipitate salicylsulphonic acid.

IX. **On Certain Albuminoid Reactions.**

We may here mention certain reactions which were incidentally obtained in working out the preceding tests.

Salkowski[1] has recorded his opinion that gelatin gives a yellowish tint with the reaction of Adamkiewicz, a colourless reaction with Millon's reagent and a yellow with the xanthoproteic reaction. Pure gelatin prepared by the method previously described from Baird and Tatlock's best French Gelatin gives a typical xanthoproteic reaction and an equally characteristic Millon's reaction. Similarly a mixture of gelatinoses and gelatin peptone gives both reactions. With the Adamkiewicz reaction I get the same results as Salkowski. Gelatin in the solid state yields a marked blue when heated with molybdic and sulphuric acids. It does not give either Liebermann's or Schültze's reaction. Pure gelatin, as stated by Hofmeister[2] but recently doubted by certain physiological chemists, gives no precipitate with mercuric chloride. It however gives the precipitate if contaminated with a trace of a proteid.

Elastoses are precipitated by mercuric chloride.

Keratin prepared as previously stated yields all the typical albuminoid reactions in a marked manner. From the preceding observations I conclude that gelatin differs in its reactions from a proteid more than any of the other albuminoids examined and from its colour play contains a more unstable group than is present in the other albuminoids. Since this colour play is destroyed on the gelatin being converted into gelatin-peptone it is not improbable that the chemical difference between gelatin and gelatin-peptone is a difference of this molecular group. This is in accord with the view expressed long ago by Brücke[3]. Gelatin in common with the other albuminoids on account of its giving the xanthoproteic and Millon's reaction may possibly contain a hydroxybenzene nucleus.

X. **On Krasser's Reaction.**

Krasser[4] found that alloxan in solution stains proteid matter a brilliant red. In alcoholic solution he also found that it changed the colour of aspargin, aspartic acid and tyrosine to a red. He concluded

[1] Salkowski. *Berlin klin. Woch.* 1885, No. 2.

[2] Hofmeister. *Zeit. für physiol. Chem.* Bd. II. S. 299.

[3] Brücke. *Loc. cit.*

[4] Krasser. *Monat. für Chem.* Bd. VII. S. 673, and *Maly's Jahresb.* Bd. XVI. S. 1.

that it probably colours red all bodies that contain the molecular group $CH_2 - CH(NH_2).COOH$.

I find in confirmation that it colours typically dried albumen, fibrin, gelatin, keratin, elastin and mucin.

Amongst proteid decomposition products and allied bodies I find that xanthine, hypoxanthine, allantoïn, cyanuric acid, theobromine and skatol all yield negative reactions, phenol yields a red-brown only after prolonged boiling. Tyrosine and metaamidobenzoic acid yield red-brown solution, glycollic acid gives a brilliant magenta, while aspartic acid gives the typical red.

It may therefore be possible that the reaction is connected with the presence of amido groups.

XI. **On Certain Reducing Reactions.**

The property of certain tissues reducing chloride of gold in the presence of formic and other acids, has long been known and used in histological processes. Axenfeld has investigated this reaction from the point of view of a physiological chemist. He found that albuminous substances when heated with auric chloride and formic acid become blue, gas being at the same time given off. I can confirm this statement and add that proteoses give a reddish-violet which is distinct from the blue given by albumen. Gelatin gives the blue reaction typically. With keratin the solution becomes blue while the undissolved fibres turn red-brown.

All these bodies if the heating be continued for five or six minutes at the boiling point reduce the auric chloride so that a mirror of gold lines the test tube.

Axenfeld[1] stated that glucose, starch, creatine, urea and uric acid give a violet, while leucine and tyrosine a blue with these reagents.

Indole also gives the violet and alloxan the blue. Skatol immediately reduces the chloride of gold which all the others do in prolonged heating giving the gold mirror.

The large number of bodies which yield this reaction makes it valueless as a chemical test for albumen, and also prevents us determining to what group, if any, the proteid reaction is due.

It also warns us to accept with caution the statements that certain groups have been identified in protoplasm by the means of reducing actions.

[1] Axenfeld. *Maly's Jahresb.* Bd. 15 S. 27.

The statement that an aldehyde group[1] exists in living vegetable protoplasm is based upon the reductions obtained as cited in Section I. by Loew and Bokorny. I have tried with living nitella which shews "streaming protoplasmic movements" typically (and thus gives an indication of the activity of its life processes) the fuchsine reaction for an aldehyde group which was performed thus:—A dilute solution of fuchsine was completely decolorized by sulphurous acid, and into this solution pieces of nitella were dropped and watched for several hours under the microscope. The streaming movements of the protoplasm continued for a considerable period but no colouration was observed.

XII. **The Reactions of Proteid Precipitates.**

Hugo Winternitz[2] has tested the colour reactions given by the precipitate produced by the addition of acetic acid and potassium ferrocyanide to a proteid. He found the precipitate gave the xanthoproteic reaction and those of Millon, Adamkiewicz, Liebermann and Schültze. It did not give Fröhde's sulphomolybdic reaction. Winternitz does not appear to have tried the reactions of the precipitate with the salts of copper and nickel, and since the previous portion of this paper has shewn that many aromatic bodies other than proteids give the reactions above cited as conclusive for a proteid it is necessary to further test the precipitate. The precipitate is washed repeatedly till all traces of the precipitant are removed. It then shews the typical colour reactions when treated with sulphates of copper, cobalt and nickel together with potash.

From these observations I conclude that a proteid on being precipitated by acetic acid and potassium ferrocyanide is but little changed and in all probability retains a hydroxybenzene nucleus and a (CONH) group in its molecule.

Since Winternitz has shewn this reaction to be extremely delicate and the above observations remove any danger of confusion with other substances this test becomes of great practical value in urine analysis.

If the precipitate is washed free of all traces of precipitant (copper sulphate being used as an indicator) the residue from burning contains

[1] For this aldehyde test see Schiff, Caro. *Bericht d. deut. chem. Gesell.* Bd. XIII. S. 2343.

[2] Winternitz. *Zeit. für physiol. Chem.* Vol. XVI. p. 439, abstract in the *Journal of the Chem. Society*, 1892, p. 1036. For the delicacy of the reaction see *Zeit. für physiol. Chem.* Vol. XV., and an abstract in *Chem. Soc. Jour.* 1891.

iron, shewing that the precipitate is due to chemical combination and not merely a coagulum like that formed on heating.

Witte's "peptone" when precipitated by this reagent shews the typical albumin colour reaction.

Foster[1] states that mercuric chloride[2], silver nitrate and lead acetate, all precipitate albumin forming insoluble compounds of variable composition with it. The precipitate may be removed by sulphuretted hydrogen and the albumin again obtained apparently unaltered in solution. Bearing this statement in mind I have tested the colour reactions in the so-called metallic albuminates.

The precipitate formed by addition of copper sulphate having been washed until all traces of the precipitant were removed (potassium ferrocyanide being the indicator) shewed the following reactions typically (1) Millon's, (2) xantho-proteic, (3) on the addition of copper sulphate and KHO the typical violet. This violet cannot be displaced by the addition of large quantities of either nickel sulphate or copper sulphate.

Similarly the precipitates obtained with silver nitrate lead acetate phosphotungstic and phosphomolybdic acids all yield the typical colour reactions with each of the proteid testing reagents.

It is therefore not improbable that the metal does not combine with either the hydroxy group in the benzene nucleus, or with the nitrogen group to which the copper, cobalt and nickel reactions are due.

XIII. **The experiments of Grimaux on coagulation[3].**

Before I was aware of Grimaux's work (see p. 352) I thought that the sulphonic group which has been demonstrated in proteids by Loew might not be unconnected with the changes of coagulation since it has a marked affinity for the metals of the calcium group, but Ringer[4] has shewn that calcium chloride has no coagulating effect on gelatin, while the older view of Hofmeister[5] that gelatin contains no sulphur has been negatived by the analyses of Gorup-Besanez[6] and of Chitten-

[1] Foster. *A Text-book of Physiology*, 4 ed. p. 703.

[2] If mercuric chloride is used as a test for albumen in urine it is particularly necessary to apply the colour test as suggested, since Grecne (*Brit. Med. Jour.* 10th May 1879), and Johnson (*Proc. Roy. Soc.* Vol. XLIII. p. 499), have emphasised the fact that *normal urine* yields a precipitate with mercuric chloride.

[3] *Loc. cit.*

[4] Ringer. This *Journal*. Vol. XII. p. 390, (1891).

[5] Hofmeister. *Zeit. für physiol. Chem.* Vol. II. p. 299.

[6] Gorup-Besanez. *Lehrbuch der physiologischen Chem.* (3rd ed.), 1874, p. 150.

den[1]. Therefore it would appear that the presence of a sulphonic group in the proteid molecule does not determine its power of coagulation.

The researches of Nencki[2], Sécretan[3], and Weyl[4] have shewn that indole and skatole are not found in the putrefaction products of gelatin, while Schützenberger[5] has pointed out that tyrosine is also absent. These observations shew that the aromatic portion of gelatin (which is demonstrated by its yielding Millon's and the xanthoproteic reaction) differs in its arrangement from the aromatic portion of proteids.

If the statements of Grimaux could be substantiated that the power of coagulation is a property of an intra-molecular anhydride of amido-benzoic acid, and as the properties assigned by him to that body coincide with those since described by many observers as characterizing proteids, we might conclude that the coagulation of proteids was intimately associated with their aromatic nucleus.

Unfortunately Grimaux has given but meagre details of his experiments, he does not state at what temperature the synthesis was carried out.

Amido-benzoic acid itself gives no reaction with copper sulphate and potash, it gives an orange with Millon's test and a pale yellow with xanthoproteic reaction. It does not clot or coagulate when heated with a few drops of calcium chloride.

When amido-benzoic acid is heated with phosphorous pentachloride (as Grimaux directs) to a temperature[6] of 125° C. by an oil bath for three or four hours a flaky substance which is not unlike dried albumin in its physical properties is formed. It becomes insoluble in water at 100° C. and as Grimaux has stated is soluble in ammonia.

Grimaux states that this solution when it has a few drops of calcic chloride added to it does not coagulate alone but coagulates on heating. Now any phosphorous pentachloride that may have been unchanged in the synthetic reaction, would when brought in contact with water form phosphoric and hydrochloric acids, and the addition of calcic chloride to

[1] Chittenden and Solley. This *Journal*. Vol. XII. p. 23.

[2] Nencki. *Jahresb. Thierch.* 1876, p. 31.

[3] Sécretan. *Archives des Sciences de la bibliothèque universelle de Genève*, 1876.

[4] Weyl. *Zeit. für physiol. Chem.* Vol. I. p. 339.

[5] Schützenberger. Article "Albuminoids" in *Wurtz' Dictionnaire de Chim. Supplément*. Vol. I. p. 58.

[6] This temperature was chosen because Schützenberger's synthetic experiments were carried out at this heat.

the ammoniacal solution would produce a white flaky precipitate of calcium phosphate which looks extremely like a proteid coagulum[1]. It therefore devolves upon Grimaux to shew that he has removed all phosphates from his solutions which in the short account of his experiments published in the *Comptes Rendus* he has neglected to do. Until he has done this, his observations are open to doubt. A detailed account of the experiments would therefore be valuable.

I can however confirm Grimaux's statement that the "Colloïde amidobenzoïque" gives a blue-violet solution with copper sulphate and potash. With Gnezda's reaction it gives a pale yellow, and with cobalt sulphate and potash a purple-brown, changing rapidly to a red-brown.

XIV. **Summary of Chief Results.**

1. The preceding observations tend to shew, that excepting Fröhde's and Axenfeld's reactions, each of the characteristic proteid tests examined corresponds to changes set up in definite molecular groups in the proteid molecule.

2. That cobalt salts and potash give delicate and distinctive colour reactions with proteids, proteoses, and peptones (proteid = heliotrope purple; proteoses, and peptone = red brown).

3. That characteristic colour reactions are given with cobalt sulphate, caustic potash and the following albuminoids, gelatin, keratin, elastin, colloid substance of the thyroid gland, and mucin.

4. That the same reagents give colour reactions with nitrogen katabolic compounds and certain bodies of allied chemical constitution, *e.g.*—biuret, alloxan, uric acid, xanthine, hypoxanthine and murexide.

5. That the colour plays obtained with gelatin are in the same spectral order as the colour plays obtained with alloxan, biuret, and allantoïn.

6. That it is not improbable that the colour reactions of proteids, albuminoids, proteoses, and peptones, with the salts of cobalt, nickel and copper together with potash are due to the chemical group (CONH) common to the former bodies.

7. That it is not improbable that the difference between a proteid

[1] The resemblance in physical property to albumen is so great that it has been substituted for that body in certain technical processes.

and a peptone, is a differenee in the atomic arrangement of this (CONH) group.

8. That when a cobalt salt has entered into the proteid moleeule, it can be easily displaced by a nickel salt and the nickel salt by a copper salt, eaeh in turn yielding its eharacteristic eolour reaetions.

9. That cobalt sulphate is a more delicate reagent for proteid testing than either nickel sulphate or copper sulphate.

10. That the eolour reactions given by metallie salts and potash with proteids and allied bodies, are not a function of the "periodic law."

11. That the chief proteid precipitates, *e.g.* those formed by the addition of mercuric chloride, silver nitrate, salicylsulphonic, phosphotungstic and phosphomolybdie aeids yield typical proteid colour reactions.

12. That nucleoalbumins behave as ordinary proteids and not as peptones in all their colour reactions.

13. That further examination of the precipitates given by certain reagents commonly used in testing urine for albumen is desirable to avoid the confusion of a foreign body with albumen.

14. That the colour tests should be applied to the precipitates given in Winternitz' and MacWilliam's tests, in which case it is difficult to mistake any other substance for albumen.

15. That the xanthoproteic reaction probably depends on a hydroxybenzene nucleus in the proteid molecule.

16. That Millon's reaction probably depends on the same "nucleus."

17. That the reactions of Liebermann and of Adamkiewicz probably depend upon the aromatic portion of the proteid molecule.

18. That Krasser's reaction may depend on an amido group present in proteids and albuminoids. That no evidenee has been found against Krasser's views on this matter.

19. That a negative result was obtained with the fuchsine aldehyde test and living vegetable protoplasm.

20. That when meta-amidobenzoic acid is heated with phosphorous pentachloride it yields a substance which gives eolour reaetions with salts of cobalt, nickel and eopper which are very similar to the eolour reactions of proteids.

21. That the experiments of Grimaux seen in the light of recent researches on the relationship of the alkaline earths and the eoagulation

of proteids would if substantiated throw considerable light on the molecular mechanism of coagulation.

I hope in a subsequent paper to investigate in a similar manner the colour reactions of Riechl, Michaïloff and Petri.

In conclusion it is my very pleasant duty to record my best thanks to Prof. Halliburton for the help, advice and kindly criticism, which he has constantly given me throughout my work.

Jan. 14, 1893.